A CYTOLOGICAL STUDY OF SECRETORY PHENOMENA IN THE SILK GLAND OF *HYPHANTRIA CUNEA*.

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INTRODUCTION.

The general anatomy and histology of the silk glands of lepidopterous larvæ have been carefully studied and described by numerous investigators beginning with the work of Helm ('76), but little purely cytological study of the cells with an aim toward giving an account of the mechanism of secretion was reported before the investigations of Maziarski ('11) and later of Nakahara ('17). Since that time, few papers have appeared based upon studies of the silk gland, with the exception of an account by Yamanouchi ('22) describing a morphological study of silk secretion.

In previous investigations of silk secretion, studies have been made only upon glands of caterpillars which spin a cocoon at a certain period in their life history. Such study is of value in observing glandular function and development during the progressive stages preparatory to and at the time of activity. In order to best study the actual process of secretion, the optimum material would naturally be found in a form which secretes actively over a comparatively long period of time.

The fall webworm, *Hyphantria cunea*, is ideally adapted for the study of active secretion, for the caterpillars live together in colonies, secreting the web continually throughout larval life in the process of securing food. This form, therefore, was selected for the present investigation and by the application of cytological methods, an attempt has been made to determine the cellular organization which is concerned with secretory phenomena in the silk gland.

Work upon this problem was for the most part carried on during 1925 and 1926, at Washington University, St. Louis,

under the direction and supervision of Prof. Caswell Grave. It is with great pleasure that I take this opportunity to express to Dr. Grave my sincere appreciation for his advice, criticisms, and valuable suggestions.

Material.

The spinning-glands of the larval *Hyphantria cunea*, like those of all lepidopteran larvæ, consist of two long tubes, easily distinguishable by their glassy appearance, situated on either side of the nerve cord and below the digestive tract. Each tube is a cylindrical, folded body, averaging between 6 and 8 mm. in length, or slightly less than the entire length of the caterpillar which averages about 1 cm. In total mounts of a spinning-gland, three arbitrary divisions may be distinguished, characterized by differences in appearance, and, in section, by structure (Text-Fig. *D*); the posterior, or secreting portion lying between the eighth and eleventh segments and terminating blindly; the middle portion or reservoir, bending posteriorly to the ninth or tenth segment, then folding anteriorly to the fifth segment; the anterior, or conducting portion extending straight forward to the head where the two tubes converge and unite to open at the apex of a median cylindrical organ, the spinneret.

Each tube consists of a single layer of epithelial cells (Imms, '25, p. 407), varying in shape and size according to position in the tubule. The cells are arranged in two longitudinal rows around a central lumen. The nucleus of each cell is characteristically branched and very irregular. Externally, the gland is covered by a thin membrane, the tunica propria, and internally, the lumen is lined by the thicker tunica intima.

The cells of the posterior region are relatively large and irregular in form and contain a greatly branched nucleus. The inner cell wall is irregular in surface contour, due to numerous drops of secretion in passage between the cell and lumen. The tunica intima is not readily distinguishable (Text-Fig. *A*, *B*). The cells composing the middle region are greatly flattened with a consequent elongation of the nucleus, and possess a clear hyalin, inner border. The cavity of the lumen has increased in diameter and is filled by secreted material (Text-Fig. *C*, *D*).

The cells of the anterior conducting portion are reduced in size, being compressed but less elongated than cells of the reservoir. The nuclei tend to be rounder and less branched, and the intima can be definitely distinguished (Text-Fig. *E*, *F*).

The tracheæ that supply the posterior and middle regions penetrate through the tunica propria into the substance of the cell. It is significant that no intracellular tracheæ are present in the anterior conducting portion of the silk glands.

Color Reactions of Silk Gland Cells with Various Stains and Fixatives.

Fixative and Stain.	Chromatin.	Nuclear Bodies.	Nucleoloid Bodies.	Mitochondria.
Benda:				
Delafield's Hæm., Acid Fuchsin..	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Blue	
Ehrlich-Biondi.....	Pink	Pink	Orange	
Iron Hæm., Erythrosin.....	Black	Black	Black	Black
Bouin:				
Delafield's Hæm., Acid Fuchsin..	Blue	Blue	Blue	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Black	
Iron Hæm., Acid Fuchsin.....	Pink	Black	Black	
Champy:				
Altmann, Acid Fuchsin, Methyl gr.....	Green	Red	Red	Red
Delafield's Hæm., Acid Fuchsin..	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Blue	
Ehrlich-Biondi.....	Pink	Red	Orange	
Iron Hæm., Acid Fuchsin.....	Black	Black	Black	Black
Gatenby:				
Delafield's Hæm., Acid Fuchsin..	Black	Black	Black	
Iron Hæm., Acid Fuchsin.....	Black	Black	Black	Black
Mann-Kopsch:				
Unstained.....	Yellow	Yellow	Yellow	
Altmann, Acid Fuchsin, Methyl gr.....	Reddish	Reddish	Brown	
Regaud:				
Altmann, Acid Fuchsin, Methyl gr.....	Green	Red	Green	Red
Iron Hæm., Erythrosin.....	Pink	Black		Black

Larval *H. cunea* furnished the entire supply of material with the exception of a few glands from *Apatella americana* and *Diacrisia virginica* which were preserved in Bouin and DaFano preparations for comparison. The age of the webworms could not be determined as they had been actively secreting for some time previous to collection as evidenced by the size of the webs. An abundant supply of material was secured from bayberry

Color Reactions of Silk Gland Cells with Various Stains and Fixatives.

Fixative and Stain.	Cytopl. Bodies.	Secretion.		Intima.	Mucoid Sub.	Cytopl.
		Outer.	Inner.			
Benda:						
Delafield's Hæm., Acid Fuchsin	Pink	Light brown	Olive	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin			Olive	Olive		Pink
Ehrlich-Biondi	Pink	Pink	Blue-gray			Green
Iron Hæm., Erythrosin	Black		Black	Pink	Pink	Pink
Bouin:						
Delafield's Hæm., Acid Fuchsin	Pink	Olive	Pink			Pink
Ehrlich's Hæm., Acid Fuchsin	Pink	Pink	Dark pink	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black	Black	Pink	Black	Pink	Pink
Champy:						
Altmann, A. Fuch., Methyl green	Red	Dark red	Light red	Green	Green	Green
Delafield's Hæm., Acid Fuchsin		Pink	Brown	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin	Bluish	Pink	Olive	Pink	Pink	Pink
Ehrlich-Biondi	Pink-green	Red-orange	Green-gray	Green	Pink	Green
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Gatenby:						
Delafield's Hæm., Acid Fuchsin		Dark brown	Light brown	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Mann-Kopsch:						
Unstained	Black		Olive	Brown	Yellow	Yellow
Altmann, A. Fuch., Methyl green	Olive	Olive	Red	Green		Green
Regaud:						
Altmann, A. Fuch., Methyl green	Red	Green	Red-green	Red		Green
Iron Hæm., Erythrosin	Black	Black	Black	Black		Pink

bushes on Nonamessett Island, Mass., between August 13 and 16, and brought into the laboratory at Woods Hole where the animals were kept supplied with food and moisture until killed.

Methods.

The caterpillars were killed by piercing the head with a pin after which they were immediately fastened in a paraffined dish containing Locke's solution, opened by a dorsal incision, and the glands removed directly with glass needles and placed in fixing fluids. Several fixing solutions were used, namely; Bouin's, Zenker's, Gilson's, for general structure, Benda's, Gatenby's modified "Flemmings" for cell inclusions, Champy's method for preserving and differentiating Golgi bodies and mitochondria, and Regaud for mitochondria.

Transverse and longitudinal sections, 4 micra in thickness, were made of the glands and stained by various methods. The results of different combinations of fixatives and stains may be seen from the chart.

Observations.

1. *The Nucleus.*

(*a*) *Historical.*—The nucleus of larval spinning-gland cells has been found to be characteristically large and greatly branched; the branches extending toward all sides of the cell. This peculiarity of shape early attracted investigators and led them to study the nucleus and its contents in an effort to find some possible relation between its unusual form and the active secretion of silk; but it was not until Korschelt in 1896 published the results of his observations upon living and fixed material that a detailed account of the nuclear content of spinning-gland cells appeared in the literature. From the results obtained through use of a modification of the Ehrlich-Biondi stain, Korschelt concluded that the larger bodies or macrosomes found in the nucleus correspond to the chromatin, and the smaller bodies, or microsomes to nucleoli. Meves ('97) used other staining reagents and came to opposite conclusions. He identified the macrosomes with the nucleoli and the microsomes with chromatin. More recent investigations have seemed to support

the conclusions of Meves (Marshall and Vorhies, '06; Vorhies, '08; Maziarski, '11; Nakahara, '17).

(*b*) *Observations.*—Within the nuclear membrane, which can usually be distinguished with little difficulty in the spinning-gland cells of *H. cunea*, bodies of two sizes are always present (except in the anterior conducting region) and with great regularity and frequency a larger, third body is also found (Fig. 5). The smallest bodies are by far the most abundant and tend to fill the entire nucleus. With certain fixatives, Bouin, Zenker, a reticulum can be distinguished, upon which are arranged these smallest bodies. This is not the case, however, after fixation in Regaud, Champy, Benda, or Gatenby, for in the middle and posterior regions of the gland the bodies always appear to be free in the nucleus, forming a compact mass of small granules, and take a basic stain. (See chart.) These bodies have been considered as chromatin. In the anterior region of the gland, these bodies are consistently present although considerably more scattered.

The second group of bodies present in the nucleus exhibits considerable variability both of size and distribution. These are the macrosomes or nucleoli of previous workers, but in this paper, they will be referred to as "nuclear bodies." It is only rarely that these nuclear bodies are found in the nuclei of the conducting cells, and when they chance to be present, are considerably smaller than similar bodies in the posterior or middle regions. No general rule can be given for the shape or dimensions of these nuclear bodies as they tend to vary greatly. Their usual shape is spherical or ellipsoid, and this is typical of their form in the middle region, but toward the posterior end of the gland where there are evidences of active secretion indicated by droplets in the lumen and on the edge of the lumen, these bodies are often elongated and irregular in shape. In size, there is variation in length between 0.5 micron and 2 micra and occasionally a few are larger. Even within the same nucleus, such variation in size is present. The bodies have no special arrangement in the nucleus but tend to be dispersed throughout the area, very often approaching the nuclear membrane. They have even been seen penetrating the nuclear wall into the cyto-

plasm. Critical observation with high magnification definitely showed nuclear bodies partly within the nucleus and partly in the cytoplasm (Fig. 4). The passage of nuclear bodies into the cytoplasm is a fact that has been described with great care by Maziarski ('11), Nakahara ('17), and earlier workers, as of regular occurrence in the spinning-gland cell; by Saguchi ('20) as occurring in nuclear function of the pancreas cell; and by Ludford ('25) in tissue cultures of fibroblasts of the rats' kidney.

The staining reaction of these nuclear bodies is of interest because of the fact that although it is more or less variable, yet it tends to show greater affinity for acid stains. (See Chart.) It has been upon reactions to stains that all the conclusions in regard to the relation between nucleus and secretion have been made, since it has been regularly found that the nuclear bodies (nucleoli) and apparently related bodies in the cytoplasm exhibit the same staining reactions.

The third class of nuclear bodies found in *H. cunea* has not been described in the literature so far as it has been possible to determine. Because of the similarity between these bodies and the true nucleoli, they will be referred to throughout this paper as "nucleoloid bodies." Meves ('97) mentions the fact that nucleoli may contain several small vacuoles, but in the figures of nucleoli in which he shows this structure, they are no larger than numerous other bodies in the nucleus. Korschelt ('97) in replying to the criticism of Meves, attempts to explain the presence of vacuoles by the hypothesis that the nucleoli or microsomes are derived from the chromatic substance by some transformation evidenced, no doubt, by these vacuoles. Marshall and Vorhies ('06) definitely state that no plasmosome or special structure is formed in the nucleus during secretion in spinning-gland cells of *Platyphylax*, but mention the fact that sometimes the nucleoli may contain vacuoles but no further significance is attached to the observation; nor did Nakahara ('17) consider as important the presence of vacuoles in nucleoli. In the nuclei of *H. cunea*, vacuolated bodies are found to be sufficiently definite to attract especial attention. After carefully measuring a large number of such bodies in all parts of the silk gland, it was found that the average diameter is about 4 micra regardless

of position in the gland. Several measured 3 micra in diameter and a very few in the more actively secreting region are as large as 5 micra or 6 micra, while in the anterior region, these bodies average about 2 micra in diameter. This size relation is constant regardless of the fixation used.

Each nucleoloid body contains two or more vacuoles or clear spaces which either do not stain or, if so, only faintly. In a section of a nucleus, usually only one of these bodies is present, rarely two (Figs. 3, 5, 6), and the frequency with which they occur in serial transverse sections 4 micra in thickness leads to the conclusion that not more than two are usually present in a nucleus. This point has been very difficult to determine accurately on account of the uncertainty in deciding where one nucleus ends and the next begins. From longitudinal sections, the evidence was more definitely in support of this conclusion. These nucleoloid bodies are either centrally or just eccentrically placed, and are never found in close approximation to the nuclear wall. Their usual shape is spherical, thus distinguishing nucleoloid bodies from nuclear bodies which tend to vary in shape. From careful study, it has appeared that the presence of nucleoloid bodies is usually associated with an increase in the number of nuclear bodies (macrosomes) either in the same section or the next in series. In the immediate vicinity of the nucleoloid body, the nuclear bodies are smaller, increasing in size toward the periphery of the nucleus (Figs. 3, 5, 6). The periphery of the nucleoloid bodies is not always regular, for often protrusions apparently in the process of pinching off may be seen (Fig. 5).

That the vacuolated nucleolus may give rise to nuclear bodies is not without precedent in observations. Saguchi ('20) in the pancreas cell, describes a very similar process which gives rise to nucleolar corpuscles: p. 355: "As regards the genesis of the nucleolar corpuscles, I am fully convinced that they are derived from the main or side nucleoli." Montgomery ('99) concludes that the nucleolus may act as a nuclear organ either of excretion or storage and that when it has enlarged to a certain extent, it constricts pieces from itself.

The nucleoloid bodies usually react more specifically to basic

stains (see chart), but occasionally with Altmann's anilin acid fuchsin, methyl green after fixation by the Regaud method, bodies can be found staining deeply green at the periphery and pink in the center.

(*c*) *Critical.*—From observations upon silk gland cells of *H. cunea*, it would seem that the bodies which migrate from the nucleus into the cytoplasm are not nucleoli, but are rather products of nuclear activity. That this is placing too great a burden upon a single small organ of the nucleus may be argued, but it need not necessarily follow that all the products of secretion are derived from the nucleoloid bodies, but rather that the latter contribute bodies which enlarge in the nucleus, pass out into the cytoplasm where they undergo further modification and later become discharged into the lumen. In order to verify the concluding part of this statement, a general survey of the cytoplasm and its contents is necessary.

2. *The Cytoplasm.*

(*a*) *Cytoplasm.*—The cytoplasmic structure of the silk gland cell as of any cell, is difficult to describe in concrete terms for it is more or less affected by fixation and the exact or true nature is hard to determine. After fixation with the Champy, Gatenby, Benda, or Regaud methods, the cytoplasm is homogeneous and granular, but after Bouin and Mann-Kopsch fixation, a definitely striated appearance may be distinguished, the striæ extending from the lumen toward the periphery. No evidence of fibrils have been found in any case. Yamanouchi ('22) definitely describes fibrillæ in the protoplasm between which are situated granules, especially toward the lumen. These, he explains, are cross sections of fibrillæ and that the entire structure is composed of minute fibrillæ arranged in rows. The usual staining reaction is acidophilic. (See chart.)

(*b*) *Granules.*—In the posterior region of the gland, throughout the cytoplasm, there appear spherical bodies either enclosed in vacuoles or apparently free in the protoplasm (Fig. 1). Maziarski ('11), Nakahara ('17), and others, noted this condition and from the fact that the bodies are often found in close proximity to the nucleus and exhibit the same staining reactions

as the nuclear bodies drew the conclusion that the cytoplasmic granules are derived from the nuclear bodies (nucleoli) by migration of the latter. Tanaka ('11) believes the fibroin, or silk core, to be a product of the gland cell substance, visible only when the secretion has accumulated to give rise to fine fibroin droplets which unite to form larger droplets. Yamanouchi ('22) likewise believes that secretion is produced in the cell.

Whether or not these cytoplasmic bodies are produced in the cell or in the nucleus, it seems to be generally concluded that they are directly connected with the products of secretion.

From observation of cytoplasmic granules in the cells of *H. cunea*, it was found that they average between 1 micron and 2 micra in diameter, or approximate the size of the nuclear bodies. The cytoplasmic granules tend to increase in size as they approach the lumen and finally, the droplets of secretion when passed into the cavity coalesce and become of considerable size (Fig. 16). Evidence of active secretion is correlated with an abundance of cytoplasmic bodies present in the cell, but not, as might be expected, with a decrease in the number of nuclear bodies present. It seems, therefore, that if the nucleus does play an active part in the formation of secretion that there is always an excess of nuclear bodies stored in the branched nucleus, and while the drain upon one part may be great, the reserve from another part of the nucleus may supply the demand.

The cytoplasmic bodies are found in all parts of the cell, but usually most abundantly between portions of a nucleus or between the nucleus and the lumen (Fig. 1). These bodies usually react to acid stains, but are generally stained black with iron hæmatoxylin. (See chart.)

That no cytoplasmic bodies are present in the middle section is difficult to determine with absolute certainty. If the nucleus with its nucleoloid bodies and nuclear bodies is responsible for secretion, surely secretion should occur in this region, for both types of bodies are present in the nuclei. Material fixed by the Gatenby method and stained with iron hæmatoxylin and acid fuchsin furnished the only indication that cytoplasmic bodies may be present in the reservoir region. The bodies took a light pink stain very similar to that of the clear, structureless substance

surrounding the silk core. These bodies were so indistinct that they may have been the result of poor technique.

Yamanouchi ('22) finds evidence that sericin, or the outer layer of the silk core, is secreted in the posterior portion of the middle region. Blanc ('89) considers that mucous is secreted in the anterior portion of the reservoir in the form of numerous granules, but this point has received little support in subsequent literature.

(*c*) *Mitochondria.*—Throughout the whole of the literature dealing with the silk glands of various lepidopteran larvæ, no reference has been found which even suggests that the workers suspected the presence of mitochondria in the cells. This may be due to the fact that no investigator has employed specific mitochondrial fixatives.

Maziarski ('11) describes certain bodies that are found occasionally in the protoplasm as having the form of short "bâtonnets," and staining deeply. These have a parallel arrangement along the shorter axis of the cell and unite with one another by fine protoplasmic fibrils. The substance of these bâtonnets is refractile and stains with acid stains. Gilson noticed these bodies and thought they were related to functional processes of the protoplasm in that they have the appearance of little tubules filled with secretory substance. Maziarski did not find these bodies consistently present in all of his material.

After fixation with Regaud, and staining with Altmann's anilin acid fuchsin, methyl green, clear-cut evidence of the existence of mitochondria in the cells of *H. cunea* has been obtained.

1. *Posterior Region.*—The mitochondria in the posterior region are very numerous, especially between portions of a nucleus and toward the lumen. In whatever part of the cell the mitochondria may appear, they always show a characteristic orientation with their longitudinal axis toward the lumen. There is no evidence that these bodies have any direct relation to the nucleus for they do not radiate from it or tend to cluster around it (Figs. 1, 2).

Their usual shape is filamentous, rod-shaped or granular. Where active secretion is taking place, long filaments are present,

averaging between 3 and 5 micra in length, and about 0.5 micron in diameter. In the more anterior portions of the gland, the mitochondria are found to be shorter, but equally numerous. Mitochondria do not appear to have any direct relation to the cytoplasmic bodies, but the latter are found situated among the mitochondrial filaments.

Material stained with iron hæmatoxylin after fixation in either the Champy or Gatenby method exhibits the presence of structures similar in size, shape and distribution to those observed with Regaud fixation (Figs. 5, 6).

2. *Middle Region.*—In the middle region, mitochondria are always present but their shape is somewhat different from that of the mitochondria in the posterior region. A gradual transition may be seen from the long filaments in the secreting region to the shorter rods in the anterior portion of this region (Fig. 7) and finally, in the true reservoir, the mitochondria become shortened to granules or spherules. The latter have the properties of mitochondria but are somewhat larger in diameter than the mitochondrial granules. It is not probable that the larger spherules represent secretion since in appearance, they more nearly resemble mitochondria than secretion bodies.

The distribution of mitochondria is fairly disperse throughout all parts of the cell, but with a tendency to be more abundant toward the lumen. No definite orientation can be distinguished in the longer rods (Fig. 12).

3. *Anterior Region.*—Bodies taking a stain characteristic of mitochondria are present in the anterior region, but these are always granular and arranged with little definite order (Figs. 8, 9).

4. *Critical.*—From the preceding observations, it is seen that mitochondria are present in the cytoplasm throughout the entire extent of the tubule. Their shape and to a less extent their number varies, however, in the three arbitrary divisions of the gland. Cowdry ('24, p. 318) suggests that mitochondria may serve to some extent as indicators of secretory polarity. In the case of silk secretion, it seems that this is truly the conclusion to be drawn. Secretion can be traced from the cell into the lumen, and mitochondria appear to be streaming toward the

lumen in a very definite way. There is no evidence that mitochondria actively produce secretion but only the indication that they may participate in the process. That mitochondria are present in the reservoir and conducting regions but that here they do not show polarity seems to indicate that mitochondria may be present to perform other functions than that of participating in secretory activity. Activity is occurring in both the middle and anterior regions, although not secretory activity. The middle portion appears to be a storage place for secretion where possible modifications of the secreted material may take place, for example, a condensation and compression. The secreted material then passes through the anterior conducting portion. Tanaka ('11) is the only investigator who has suggested any possibility of the existence of secretory function in the anterior region, and this probably only in the embryonic stages. The usual interpretation is that this region serves as a duct for the passage of secretion. In this case, the presence of mitochondria would seem to indicate that some activity exists within the cell, possibly of general metabolic nature.

(*d*) *Golgi Bodies.*—In preparations of glands fixed by the Mann-Kopsch, and by the Champy method, no structures have been found which could be interpreted as true Golgi bodies. In silk gland cells of *H. cunea* impregnated with osmic acid, numerous fine dark granules appear (Fig. 11), that are distributed very abundantly along the periphery of the cell-body and scattered more generally toward the lumen. These fine granules are present in all regions of the gland and always characteristically more abundant toward the periphery of the cell. In the extreme posterior tip of the gland, the granules appear grouped as a dense border which gradually becomes more disperse toward the lumen. In cells of the secreting region, groups of large, black bodies, spherical in shape and usually situated toward or near the lumen or the periphery are common and may be followed through several sections of a series.

Miss Murray ('26) describes a condition in the follicle cells of the cricket which seems quite similar and her figures compare closely with the condition found in the spinning-gland cell. With regard to her observations, she states (p. 223) that "In

the proximal region of some of these cells (follicle cells) there has been seen a nebulous structure composed of very finely divided black particles, which might be considered to indicate an excessive deposition of fat in the tissue. This fine emulsion might conceivably be coarsened at the boundary of the cell and coalesce into fatty globules."

In silk gland cells of *A. americana* and *D. virginica* which were fixed with DaFano's modification of the Cajal method, the same condition was found, but in these cases, there is a greater tendency toward an arrangement of the granules in rows, thus presenting the appearance of striations extending from the lumen toward the periphery, and likewise a greater grouping of granules around the edge of the cell toward the lumen. These bodies tend to concentrate along all sections of tracheæ, thus outlining the course of tracheids very definitely. In these two species, it must be remembered, active secretion of silk only visibly appears at the time of forming the cocoon.

It seems possible that these fine spherical granules may represent by products of cellular activity that are being eliminated. Their condensation along the tracheids and the proximal border of the cell may indicate that such may be the case. In the two species in which the process of secretion is only in the preparatory stage, an accumulation of waste products may be imagined to be occurring in the cell.

3. *The Membranes.*

(*a*) *Historical.*—Before the work of Helm ('76), the existence of a tunica propria, or external membrane, and a tunica intima, or internal membrane was known and had been described, but little was known concerning their structure or function. The tunica propria was quickly dismissed by Helm and those following him as a structureless membrane fitting very closely to the basal membrane throughout the entire extent of the gland that resists to a considerable degree the penetration of fixing fluids. But the tunica intima could not be so easily defined. Helm describes its structure in the anterior region as containing fine radial tubes which he refers to as "Porencanale." The entire intima, according to his observations, represents a cuticular

layer which is a modified secretion of the outer body wall and is, in consequence, shed at each moult. The fine canaliculi are arranged perpendicular to the surface and are interpreted to serve as conduction paths through the cuticular intima for the liquid secreted by the cells to the lumen. Blanc ('89) working on the silk gland cells of *Bombyx mori* concluded that the intima was a chitinous structure composed of very fine parallel threads encircling the lumen at intervals of from 3–4 micra and showing occasional anastomosis. Gilson ('94) confirmed the observations of Blanc. Maziarski ('11) describing the intima, pictures the structure as a thin membrane composed of a great number of filaments passing in spiral lines around the lumen of the canal and joined by fine transverse fibrillæ, thus producing the appearance of a network of threads. These filaments are described as staining deeply with iron hæmatoxylin and having the appearance of rigid fibrils located in the protoplasm of the cell nearest the lumen, and were thought to resemble organs of support but not to serve as passageways for substances elaborated in the cells.

Tanaka ('11) describes the intima as a striated, filamentous-appearing structure of chitinous nature, thickest in the anterior division and thin in the middle region. The filamentous striations appear to have a parallel arrangement except toward the posterior region where anastomosis may occur. This chitinous substance which gives rise to the intima is thought by Tanaka to be secreted in the anterior region and never to undergo ecdysis, but rather is added to in thickness with age. This is (p. 16), he concludes, "a fact which goes a long way in proving the secretion of this chitinous substance throughout the whole larval life."

Yamanouchi ('22) found the intima to be a glassy-like cuticular layer most conspicuously present in the anterior region where it has a thickness twice that in the other parts of the gland. This structure appears to be hyalin in nature, reacting to stains very similarly to sericin but more intensely in the anterior portion of the middle region, this structure is not homogeneous but gives the appearance of containing concentric bands whereby it is possible to distinguish two or three layers of intima. This arrangement in layers disappears anteriorly.

(*b*) *Observation.*—In the gland of *H. cunea* after fixation by the Regaud method, the presence of a clear, hyalin layer of material between lumen and cell-wall is found to be quite generally present in both the middle and anterior regions. (Figs. 7, 8, 9, 12.) This layer is always found to be very closely connected with the cell-wall, and yet to be perfectly distinct from the protoplasm. Oftentimes large vacuoles may be seen in the structure (Fig. 7), but these are always on the edge toward the cell and may be explained as the result of shrinkage at the time of fixation. This vacuolated condition occurs with greater frequency in the posterior portion of the middle region where the layer is thicker and less regular in form.

Posteriorly, in the secreting region, no indication of an intima is found except for the presence of a thin membrane along the inner edge of the cells. The cells usually have an irregular surface on the side toward the lumen, the protoplasm seeming to be in very close connection with the cavity (Fig. 2), but after fixation with Champy, Gatenby, or Benda preparations, this membrane is quite definite (Fig. 1).

Toward the more anterior portion of the middle region, fibrillæ may be seen extending through the clear hyalin substance and around the lumen. In longitudinal sections through the edge of the lumen, these structures appear to extend from the edge of the cell on one side of the lumen to the cell on the other side (Fig. 10), and to take their origin along the margin of the protoplasm. These fibrillæ stain darkly with iron hæmatoxylin and dark red with Altmann's acid fuchsin after fixation by the Champy method. In general, the fibrillæ tend to be arranged in parallel series and some evidence has been found of slight anastomosis between fibrillæ in the more posterior region. After the tube has become narrowed to form the conducting portion, fibrillæ disappear leaving the intima clear and structureless.

(*c*) *Critical.*—The fact that the intima is present in the anterior and middle portions of the gland as a thickened layer seems to indicate that this structure is not connected with active secretion but must serve some function related to conduction of secreted products to the outside. A copious supply of secretion is being poured continually into the reservoir from the posterior

region. To strengthen the cell against the resulting pressure and to prevent it from distortion, a thin layer of hyalin material might be conceived to serve as a support. At the point in the tube where it begins to narrow anteriorly, the pressure becomes greater and more support is necessary. That fibrillæ should appear in the transition region between the middle and anterior regions, is, therefore, to be expected. It is quite possible, moreover, that these fibrillæ may be more than merely supporting structures. Their shape and position around the lumen seems to indicate that they may have a contractile function, serving, perhaps, as a simple musculature in pushing the products of secretion into the long conducting tube. It is evident that some regulatory mechanism is necessary to prevent the small tube from becoming clogged by the ever accumulating secretion, and likewise, to regulate the amount of material passing through in order to produce a silk thread of uniform diameter.

4. *The Secretion.*

(*a*) *Historical.*—The morphological appearance of the secreted material has received considerably more attention throughout the literature than the mechanism of the secretion process, and yet little is definitely known about the real nature of the secreted substance. Staining reactions have been used almost exclusively as criteria upon which to base conclusions. Little detailed work has been done upon the actual chemical composition of the silk fiber further than a general analysis of the chemical components, the result of which has been to show that the fiber is made up of two slightly different substances; an outer, acidophilic layer of *sericin*, and a central core of basophilic *fibroin*. The former is quite similar in composition to the latter except that, according to Blanc ('89), it contains more oxygen, dissolves more readily in alkaline solutions and is more granular in appearance.

Tanaka ('11, p. 18–20) has reviewed very completely the results of previous workers and has arranged their views concerning the origin of fibroin and sericin into four principal groups. He then attempts to show that these theories have failed to satisfy all the conditions and proceeds to modify and combine parts of each theory into one more in accord with

his own conclusions which he states as follows: "The fibroin is produced by virtue of the physiological function of the gland cells, without reference to any part of the gland tube; the sericin is, in its formation from fibroin, quite indifferent from the physiological function of the cells; on the contrary this transformation goes on merely physico-chemically."

Maziarski ('11) in his enthusiasm for the nuclear origin of secretory products, states that the nucleoli which leave the nucleus become the fibroin and that chromatin, likewise, may be extruded into the protoplasm, there to undergo quite extended changes and finally pass out into the lumen as the sericin or gummy covering of the silk thread. This idea was not entirely new, for Vorhies ('08) had suggested that chromatin material may have a secretory function in addition to serving as the "bearer of heredity."

Nakahara ('11) recognizes only the formation of silk from transformed nucleoli, but Yamanouchi ('22) describes the production of sericin and fibroin in great detail and concludes that fibroin secretion takes place only in the posterior region, but that sericin secretion occurs in the posterior and middle parts of the middle region. The two kinds of secretion appear alternately and, due to the fact that each kind tends to remain separate, two layers are formed, distinguishable by their different staining reactions.

Blanc ('89) has described a third product of secretion, called by him "*mucoidine*," which envelops the silk fiber and stains more intensely with methyl green than either the fibroin or sericin. This substance, he thought, is formed in the anterior portion of the reservoir and may serve the function of a lubricant, thus facilitating the passage of the silk.

(*b*) *Observations.*—From a comparative study of silk gland cells prepared in different fixatives, it is evident that there exist in the lumen, substances which show different types of reactions to stains. After Regaud fixation, the silk thread appears to be perfectly homogeneous throughout its entire extent except in the anterior region of the gland where a slightly darker band may be distinguished around the thread. (Fig. 9.) The secretion found in the posterior and middle portions takes a reddish-blue stain

with Altmann's acid fuchsin, methyl green, and in the anterior portion, is decidedly green. The intima takes a bright red stain and appears to be a homogeneous, hyalin substance.

The condition of the secretion product after fixation with Champy, Benda, or Gatenby solutions is considerably different and leads to doubt concerning the identity of the various structures present in the lumen. With iron hæmatoxylin and acid fuchsin, the core of secretion is stained black. In the middle region, the secretion does not fill the lumen as is the case after fixation with Regaud, but between the secretion and the intima, now distinguished as a thin black membrane at the cell border, there is present a compact mass of finely granular substance that takes the acid stain. This substance, however, is stained light green in material fixed by the Champy, and stained by the Altmann method. Usually, the material appears to be homogeneous, but after Benda, and sometimes after Champy fixation, the substance is arranged in thread-like strands that extend from the cell-wall toward the secretion. That this band of substance is separate from the core of secretion is quite evident, for the secretion may be found entirely removed from the substance and be free in the lumen. The substance is never free from the edge of the gland cell. It would seem that either the amount of secretion present is determined by the width of this band which fills the remainder of the lumen or that the size of the band of substance is dependent upon the amount of secretion present, since after fixation, little or no shrinkage apparently occurs and the core of silk is contained in the center of the lumen closely surrounded by a band of this substance. The former hypothesis seems more probable since some regulatory mechanism for producing a thread of uniformly decreasing dimensions must be present.

Material fixed by the Champy, and stained with the Altmann method affords evidence of the presence of two substances in the thread. Secreted substance in the posterior portion of the gland stains light pink on the periphery while the center is either dark pink or green. This condition is not seen as sections are selected in the more anterior part of the gland. After fixation of the gland in Bouin, the silk thread exhibits two

distinct staining reactions. The core stains a clear pink and the periphery forms a black cortex around this core. This reaction of the secreted substance is consistent throughout the tube.

In silk glands of *D. virginica* fixed in Bouin and stained in iron hæmatoxylin and acid fuchsin, the silk thread has a central core of clear pink substance surrounded by a black peripheral cortex. A wide region filled with a reticulum or meshwork of fine pink granules surrounds the thread. Bordering this region and extending to the inner cell-wall, a wide compact band of fine granular substance arranged in concentric rings occurs. Throughout this band, numerous large drops of black substance appear which probably represent secretion material. By comparison of the condition just described, with that found in *H. cunea*, and also with conditions described by Yamanouchi, it appears that some difference exists between the type of gland which secretes continuously and that which secretes at definite periods only. The presence of two diverse staining reactions in the thread seems to indicate that two substances are consistently coexistent in the two different types of silk gland cell, but the exact relationship between these two substances has not been determined with certainty.

(*c*) *Critical.*—The question as to the identity of the substances within the lumen has presented a problem which cannot be definitely settled without further study both of preserved material from a variety of caterpillars and also of living material. Observation of the material at hand seems to show that in the posterior portion of the gland, the secreted substance is usually homogeneous, and this is likewise true in the middle region. But some modification of the secreted substance takes place in the anterior conducting region which gives the silk thread the appearance of being composed of two substances.

It is evident that even in forms which secrete silk during the entire larval life, the process is not a continuous one, for often within the lumen of the silk gland, the end of one thread can be seen and, close upon the posterior part of this thread, but separated from it by the substance filling the lumen, the end of another discrete cylinder of secretion begins (Fig. 7).

The substance which fills all available space in the lumen not occupied by silk secretion, may well be similar in its properties to mucus, lubricating the tube for the passage of the silk fiber. No origin for this substance has been found. It does not appear to be secreted by the cells of the gland, nor does it seem to be a dislodged part of the bodies of the cells.

Discussion.

1. *Relation of the Nucleus to Secretion.*

The nucleus and cytoplasm of the cells of the silk gland are closely associated in the production of the silk secretion. By many investigators, the nucleus has been considered the center of secretory activity in that the nucleoli migrate from the nucleus into the cytoplasm and there undergo modification. Study of the silk gland cells of *H. cunea* has given further indication of nuclear participation in silk production through the presence of nucleoloid bodies. These nucleoloid bodies do not themselves pass into the cytoplasm but appear to constrict pieces from their periphery that increase in size within the nucleus, finally passing through the nuclear wall into the cytoplasm where further modification of their substance occurs.

2. *Relation of Mitochondria to Secretion.*

Mitochondria having a characteristic arrangement in the cytoplasm of the secreting region are present in great numbers. It is quite possible that they take some part during the transitory stage when the secretion droplets are present in the cytoplasm, in modifying the material and transmitting it to the lumen. Mitochondria may act as catalysts, as suggested by Emberger ('25) or they may take a more direct part in secretion, but their presence and orientation toward the lumen seem to indicate that they are not passive cytoplasmic inclusions. Mitochondria are present throughout the extent of the gland but in the middle and anterior portions, they are shorter than in the secretory portion and show little definite orientation. In these portions, they may aid in the process of conduction and in general metabolic activity.

3. *The Nature of the Secretion.*

The column of silk secretion is apparently composed of two slightly different substances as evidenced by diverse staining reactions, but further study is necessary before the exact origin and nature of each is determined.

Since in the posterior region, the silk thread appears to be a homogeneous cylinder when preserved in several different fixatives, it would seem that there is but one kind of substance secreted and that this substance undergoes partial modification, possibly oxidation, during its progress down the tube.

A substance similar to mucous appears in the lumen as an envelop surrounding the silk column, but no account of its origin can be given. In life, it is evidently a fluid substance because it is found to be present wherever a break in the continuity of the fiber occurs. The distinction between this substance that is finely granular in appearance after certain fixations and the clear hyalin substance present in the same relative position, following fixation in Regaud's solution, cannot be determined. The latter substance has been considered as composing the intima but the former cannot be so accounted for.

Summary.

1. In the silk gland of *Hyphantria cunea*, three regions are distinguishable; a posterior, or secreting region; a middle region, or reservoir; and an anterior, or conducting region.

2. The nuclei of silk gland cells are greatly branched in the posterior region, but tend to be more flattened and less branched in the more anterior regions.

3. Three types of bodies are found to be present in each nucleus; the small basophilic granules, or chromatin; the intermediate acidophilic bodies generally termed nucleoli, but here called nuclear bodies; the relatively large, spherical body containing vacuoles and corresponding to a true nucleolus, hence called a "nucleoloid body."

4. The nucleoloid bodies appear to give rise to the nuclear bodies which pass out of the nucleus into the cytoplasm.

5. Cytoplasmic granules exhibit the same staining reactions as nuclear bodies and are approximately the same in size when they occur in the region of the nucleus.

6. Mitochondria are present in all parts of the gland, but only in the posterior region do they show definite orientation.

7. Two membranes are present along the course of the gland, and external tunica propria, and an internal tunica intima.

8. Two substances regularly appear to constitute the silk thread in the anterior region. This condition is found to continue more posteriorly after fixation with the Champy, Gatenby, Benda, and Bouin methods.

9. A substance similar to mucous surrounds the silk thread in the middle region but does not extend into the anterior portion. The origin of this substance has not been determined.

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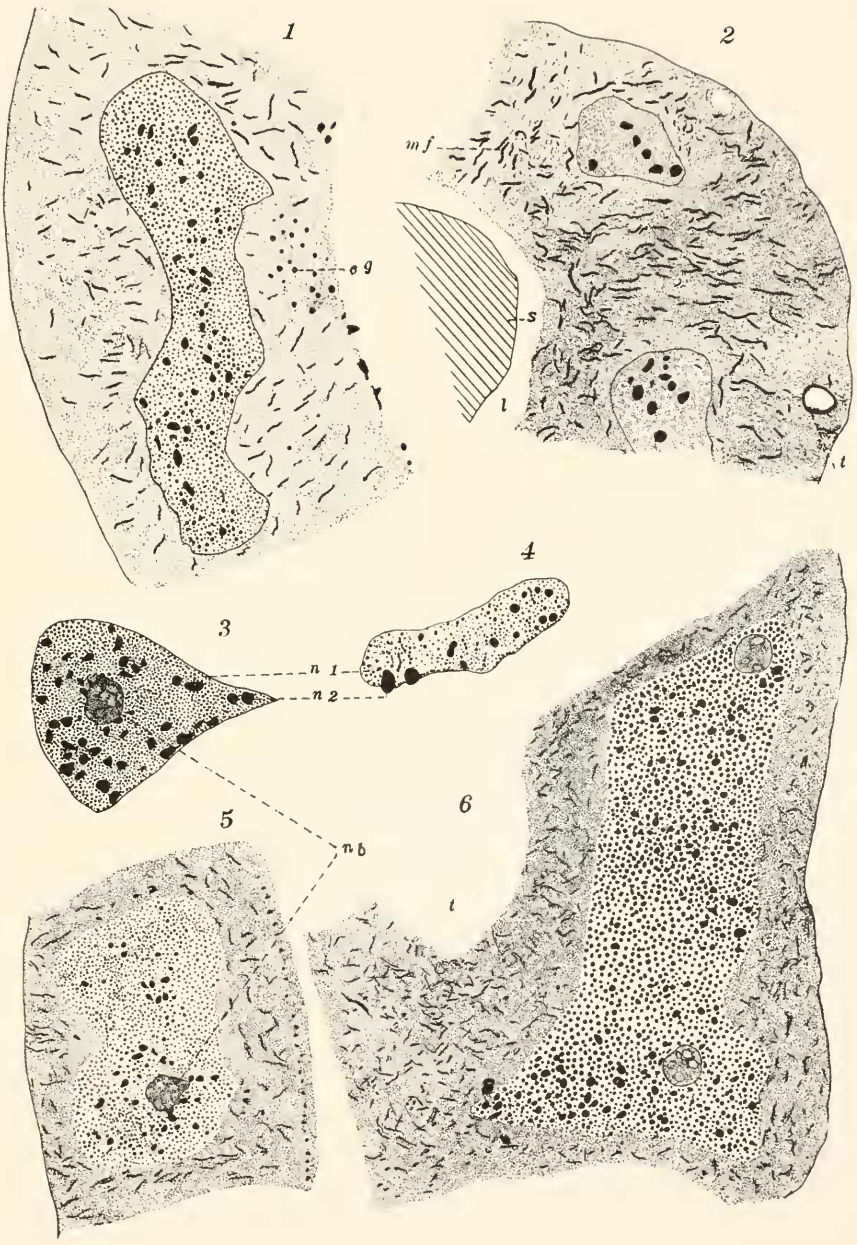
EXPLANATION OF PLATES.

Plate I.

Fig. *1*. Diagrammatic outline of silk gland of *H. cunea*. Anterior, middle, and posterior regions of gland numbered 1, 2, and 3 respectively. Letters correspond to sections taken at these levels.

Figs. *A–F*. Camera lucida outlines of sections from the three regions of a tubule fixed by the Regaud method. Cross sections cut 4 micra in thickness; magnification, oc. 10 ×, obj. 4 mm.

Note: Stippling represents cytoplasm; solid lines represent secretion; broken lines represent intima.



ELIZABETH KINNEY.

Plate II.

All figures made from sections cut 4 micra in thickness, and drawn with camera lucida, magnification, oc. 12.5 ×, obj. 1.8 mm., oil immersion.

Fig. 1. Section of cell from posterior region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Cross section. Cytoplasmic granules appear to be grouping toward lumen.

Fig. 2. Section of cell from secreting region of tubule fixed by Regaud method and stained with acid fuchsin, methyl green. Cross section. Mitochondria show definite orientation toward lumen.

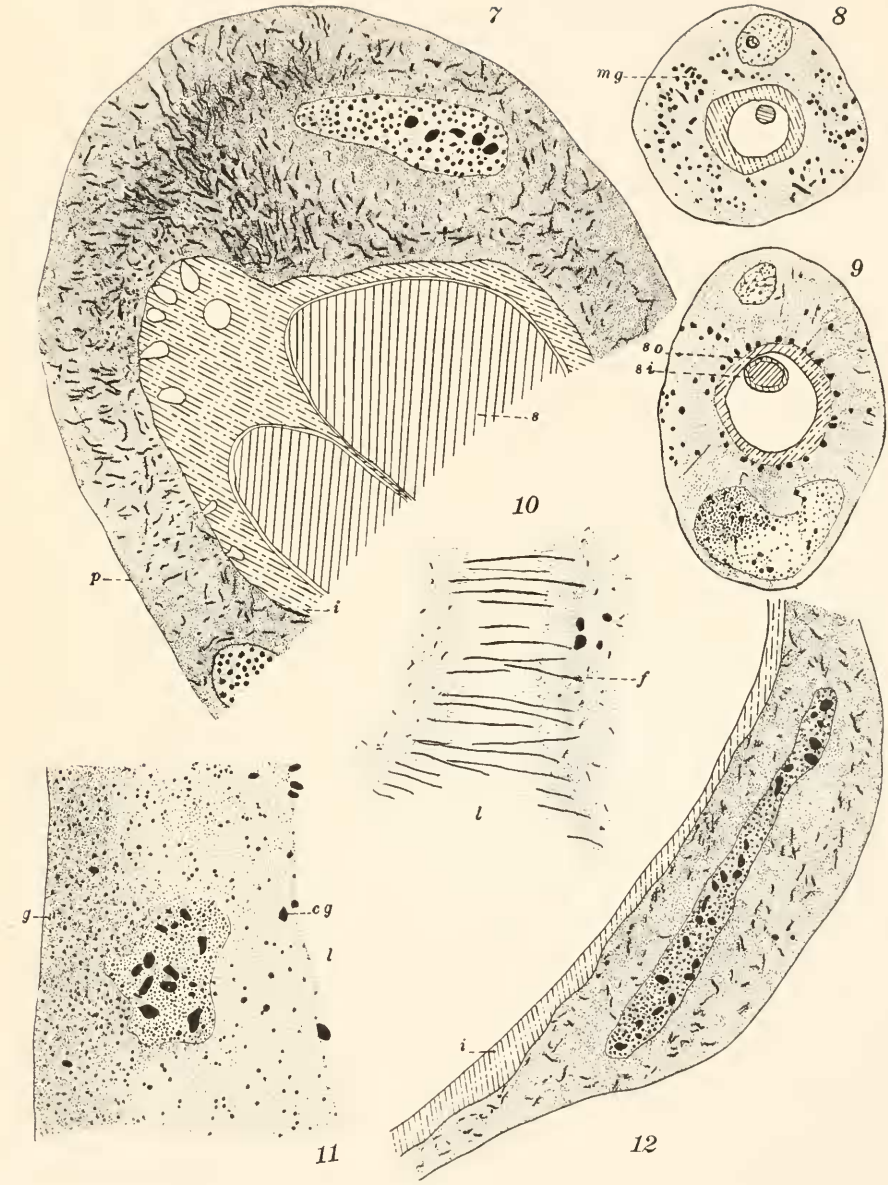
Fig. 3. A section of a nucleus from material fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. The nucleoloid body is surrounded by nuclear bodies that are smaller than those toward the nuclear wall.

Fig. 4. Section of nucleus from material fixed by the Gatenby method and stained with iron hæmatoxylin, acid fuchsin. Two nuclear bodies are in the process of passing through the nuclear wall into the cytoplasm.

Fig. 5. Longitudinal section of cell from posterior region of gland fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Nucleoloid body in process of constricting pieces from periphery.

Fig. 6. Longitudinal section of cell showing two nucleoloid bodies present in nucleus. Regaud fixation.

Symbols: *cg.* cytoplasmic granules; *l.* lumen; *mf.* mitochondrial filaments; *n1.* chromatin; *n2.* nuclear bodies; *nb.* nucleoloid body; *s.* secretion.



ELIZABETH KINNEY.

Plate III.

Fig. 7. Section of cell taken from posterior part of middle region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Cross section. Hyalin layer appears irregular and contains vacuoles along the border toward cell. Mitochondria slightly shorter. Two columns of secretion chance to be present at this level.

Fig. 8. Cross section of tubule from anterior conducting region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Mitochondria present as granules. Intima definite.

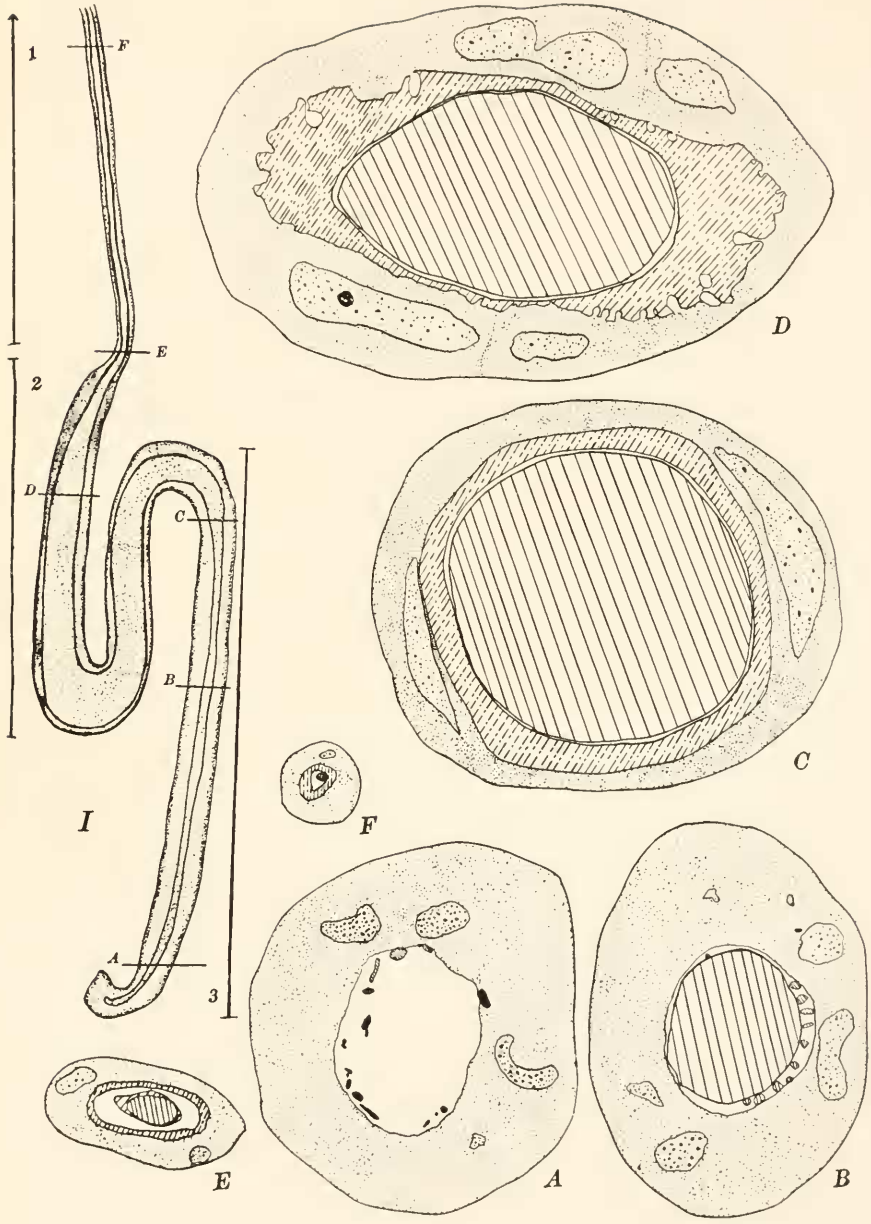
Fig. 9. Cross section from anterior conducting region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Mitochondria appear as granules. Intima well defined, two types of secretion present.

Fig. 10. Longitudinal section of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Section taken from anterior part of the middle region at edge of lumen. Fibers appear extending from the edge of one cell to the edge of the cell opposite.

Fig. 11. Longitudinal section of cell from secreting region of tubule fixed by the Mann-Kopsch method, unstained. Numerous fine black granules fill cytoplasm and tend to concentrate toward the outer cell boundary.

Fig. 12. Cross section of cell from middle region of tubule fixed by the Regaud method, stained with acid fuchsin, methyl green. Mitochondria are shorter and less definitely orientated. Clear hyalin intima present along the edge toward lumen.

Symbols: f., fibers; *g.*, granules; *mg.*, mitochondrial granules; *p.*, tunica propria; *si.*, inner layer of secretion; *so.*, outer layer of secretion.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THE LINKAGE DISTURBANCE INVOLVED IN THE CHROMOSOME TRANSLOCATION I. OF DROSOPHILA, AND ITS PROBABLE SIGNIFICANCE.

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The chromosomal abnormality in *Drosophila* known as "translocation I." was found by Bridges in 1917, and was first referred to by Morgan (1919); a preliminary account of it was given by Bridges in 1923. The case is a very remarkable one, unique in the literature to date, and it raises certain new problems concerning the arrangement which genes may have in a chromosome as well as concerning the changes in arrangement which they may undergo. The abnormality arose by a portion of the right hand end of chromosome II. breaking off and becoming attached near the middle of the right half of chromosome III. Bridges' data showed the size of the transposed piece and its approximate point of attachment on the third chromosome. It was known that the break in chromosome II. was between arc (ar) and plexus (pl), making the fragment about 8 units long, and that the transposed piece was attached somewhere between ebony (e) and rough (ro) in chromosome III. The deficient second chromosome was known as "Pale," from its effect on eosin-eyed flies, and the deficiency was lethal unless accompanied by translocation.

In the hope of getting further light on the chromosome change involved, the writer undertook to test more extensively the third chromosome linkage values in flies which carried the translocation. I wish to take this opportunity to express my indebtedness to Dr. H. J. Muller, who suggested the work, and whose aid and suggestions have made it possible. In these experiments the homozygous as well as the heterozygous condition of translocation was studied, and all of the left hand end of Chromosome III. was under genetic observation. Bridges had already shown that crossing over was greatly reduced in the region close to the locus of translocation; but his data included

only flies heterozygous for the transposed piece. The comparison of the homozygous and the heterozygous conditions with each other is of especial interest because of its bearing on the problem of the mode of attachment of the second chromosome fragment, as will be shown later.

The third chromosome mutant genes used in the experiments are, in their order from left to right along the chromosome: roughoid (ru), hairy (h), scarlet (st), pink (p), spineless (ss), delta (Δ), hairless (H), ebony (e), rough (ro), and claret (ca). Their locations are shown in the map of Fig. 1. Translocation is represented by Tr, and the deficient second chromosome by Pl. Curly (Cy) is a second chromosome gene used in balancing stocks. The linkage in flies homozygous for Tr was tested in two experiments. The summarized results for these crosses are given below, in Tables I. and II.

TABLE I.

$\frac{Cy}{Pl}\ \frac{\Delta\ H\ e\ Tr}{ru\ h\ st\ p\ ss\quad\quad Tr}$ ♀ × ru h st p ss e ♂.

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
285	202	45	86	61	10	1,097
26.0%	18.4%	4.1%	7.8%	5.6%	.9%	

TABLE II.

$\frac{Cy}{Pl}\ \frac{\Delta\ H\ e\ Tr}{Tr\ ro\ ca}$ ♀ × e ro ca ♂.

Regional Crossover Frequencies.				Total.
Δ–H.	H–e.	e–ro.	ro–ca.	
8	13	27	16	463
1.7%	2.8%	5.9%	3.5%	

These figures show a remarkable drop below normal in the crossover values as the locus of translocation is approached. This may be seen by comparing their map values (see Fig. 1,

b and *c*) with the normal third chromosome map (Fig. 1, *a*). The map length of the left-hand third will be seen to be slightly increased, but the genes between pink and claret are crowded much closer together. There is an apparent lengthening of the

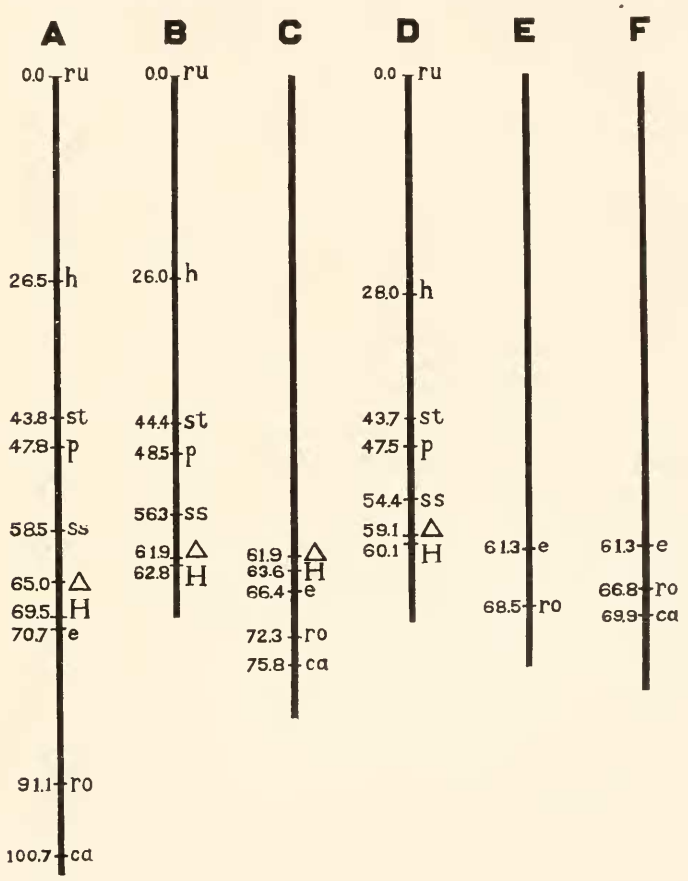


FIG. 1. (*a*) The normal third chromosome map. (*b*) and (*c*) Maps of third chromosome homozygous for translocation. (*d*), (*e*) and (*f*) Maps of third chromosome heterozygous for translocation.

distance between hairless and ebony, but this distance is so short, and the probable error so large, that the increase is probably not significant. Between ebony and rough, the region where the second chromosome fragment is attached, the per cent. of crossing over is less than one third of the normal value. These

relations are shown in Fig. 2, *a*, which shows the ratios of the values obtained in these experiments to the standard values of each of the major regions studied.

At the same time that these crosses were being made, the linkage in flies heterozygous for the abnormality was being tested. The same stocks were used in making up the crosses, and the characters used were introduced into the cross in as near the same way as possible. Both the homozygous and heterozygous lines were kept under the same conditions, so that any possible variations in viability would affect both lines alike and allow us to draw valid comparisons between them. As will be seen in Tables III. and IV., the results were almost identical with those of the first tests.

TABLE III.

$$\frac{Pl}{} \quad \frac{\Delta\ H\ e\ Tr}{ru\ h\ st\ p\ ss \qquad e} \ \female \times ru\ h\ st\ p\ ss\ e\ \male.$$

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
162	91	22	40	27	6	578
28.0%	15.7%	3.8%	6.9%	4.7%	1.0%	

TABLE IV.

$$\frac{Pl}{} \quad \frac{ru\ h\ st\ p\ ss \quad Tr}{p\ ss\ e \qquad ro} \ \female \times p\ ss\ e\ ro\ \male.$$

Crossovers.		Total Flies.
(ru h st) p ss e	(ru h st) p ss ro	
42	41	
Total Crossovers 83		1,155
7.2%		

In Table V. are results for a test involving the ebony-rough and rough-claret distances. Only those flies which did not show delta hairless could be used in computing crossover values in this experiment. This cross also gave data on the exact location of translocation, as will be discussed later.

TABLE V.

$$\frac{Cy\ ru\ h\ st\ p\ ss}{e} \quad \frac{Tr}{ro\ ca}\ \text{♀} \times \frac{Cy}{Pl} \quad \frac{\Delta\ H\ e\ Tr}{e\ \ ro\ ca}\ \text{♂}.$$

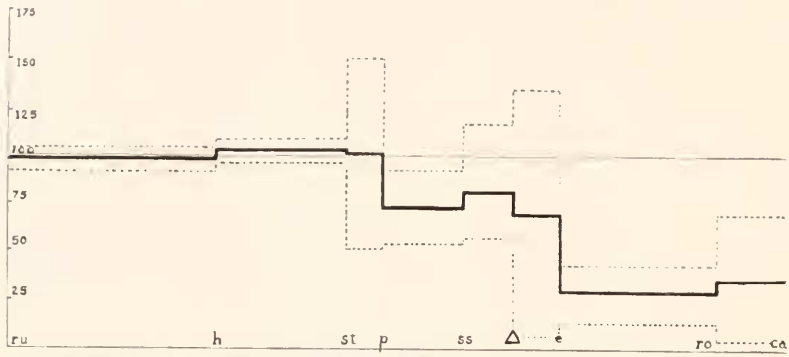
Regional Crossover Frequencies.		Total Utilizable Count.
e–ro.	ro–ca.	
50	28	902
5.5%	3.1%	

The results given in the last three tables are summed up in the chromosome maps shown in Fig. 1, *d*, *e*, and *f*, and Fig. 2, *b* shows the ratios of these values to the standard values. It will be seen on comparing these results with those based on the homozygous Tr flies that there is no noticeable difference in the crossover effects produced by the homozygous and by the heterozygous conditions.

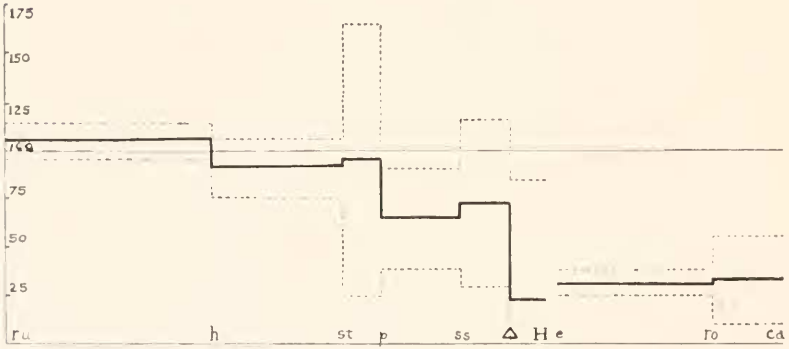
The chief significance of the results probably lies in their bearing on the mode of attachment of the translocation. There are two conceivable ways in which the translocated piece might be attached between ebony and rough: it might be interpolated within this length, thus causing a prolongation of the thread between ebony and rough, or it might be attached somehow to the side of the original thread, thus resulting in a configuration of genes and of chromatin different from what had previously been regarded as normal, and not entirely in one line. The present results clearly disprove the first alternative, for if it were true, crossing over between ebony and rough should be increased when the flies were homozygous for the interpolated fragment. We must therefore conclude that the chromosome carrying translocation is branched or doubled in the region where the latter is attached.

The second point of significance is that the presence of translocation does not reduce crossing over throughout the chromosome. Beginning at the left-hand end of the chromosome, we find that the number of crossovers is practically normal until we reach the locus of pink, one third of the distance along the chromosome. From this point on, the map distance is markedly

shortened until the locus of translocation is reached. In this interval, between ebony and rough, the per cent. of crossing over is less than one third that of the normal. Between rough and



HOMOZYGOUS TRANSLOCATION



HETEROZYGOUS TRANSLOCATION

FIG. 2. Chart showing the ratios (in percentages) of the observed crossover values to the standard. The abscissa represents the length of the III. chromosome from roughoid to claret; the ordinate represents the percentage which the observed crossovers form of the standard value in each region. The heavy line represents the percentage observed; the dotted lines mark the limits of the probable error of this percentage, calculated for each interval.

claret the amount of crossing over rises again, approaching half the normal value. The number of flies involved was not large enough to yield reliable data on the coincidences, but as far as they went they agreed well with the normal.

If crossing over takes place at a stage in synapsis in which the chromosomal threads are twisted about each other, we can readily see why the presence of the attached fragment in either one or both of the third chromosomes should produce the effects that it does. Either by stiffening the threads so that they could not twist as tightly as in the rest of the chromosome, or by an actual interference with the loops, the transposed piece should tend to prevent the close association between the homologous chromosomes necessary for that interchange of genes which constitutes crossing over. This interference would produce its maximum effect at the point of attachment, and the effect would decrease to either side. This gradual decrease in the interference is well shown to the left of ebony. The locus of translocation is too close to the right-hand end of the chromosome to show any marked diminution of its effect toward that side. The rough-claret crossovers are, however, nearer to the normal percentage than those in the ebony-rough interval.

In the cross shown in Table V., 36 crossovers between ebony and rough were observed in which it was possible to follow the behavior of the translocation. The results are shown below.

TABLE VI.

$$\frac{\text{Cy}}{\text{Pl}}\ \frac{\text{ru h st p ss}\quad \text{Tr}}{\text{e}\qquad \text{ro ca}}\ \text{♀} \times \frac{\text{Cy}}{\text{Pl}}\ \frac{\Delta\ \text{H e Tr}}{\text{e}\qquad \text{ro ca}}\ \text{♂}.$$

Crossovers between (e) and (Tr) $\frac{\text{Pl}}{\text{e Tr}}\ \frac{\text{e}}{\text{ro ca}}$	Crossovers between (Tr) and (ro) $\frac{\text{Pl}}{\text{(ru h st p ss)}}\ \frac{\text{e}}{\text{Tr ro (ca)}}\ \frac{\text{ro ca}}{}$
11	25
36	

Translocation is, according to these figures, located at approximately 11/36 of the distance between ebony and rough, or at 76.9 on the chromosome map. The data given in Bridges and Morgan's monograph on the III. chromosome characters also show translocation to be between ebony and rough, but their figures show the locus of translocation to be closer to rough than to ebony. Also, the amount of crossing over in the ebony-

rough interval is even lower than in the experiments here reported. These differences may be due to a differential viability in the two cases; there may also be differences in genes influencing crossing over, but such differences in ratios do not affect the principal conclusion of this paper. The important point is that the effect of the translocation is the same whether it is heterozygous or homozygous.

Summary.

1. The chromosomal abnormality in *Drosophila*, known as translocation I., is located at approximately 76.9 on the third chromosome.

2. The presence of translocation does not affect the crossover values for the first 45 units in the left-hand end of the chromosome. To the right of this point, the per cent. of crossing over progressively decreases as the locus of translocation is approached, reaching its minimum of less than one third normal value in the region between ebony and rough.

3. The same results are obtained no matter whether the translocation is homozygous or heterozygous.

4. The fact that the results are the same in both heterozygous and homozygous flies shows that the attached fragment is not interpolated in the third chromosome, but is attached to its side. The resulting chromosome is thus of a type which has hitherto not been reported: that is, one in which the genes are not in a single linear series.

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THE CHROMOSOMES OF THE CHICK SOMA.

ROBERT T. HANCE,

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The accumulated evidence in favor of the chromosomes being in some way concerned with the determination and control of the somatic characters has become so impressive as to be almost unassailable despite the possible objection of a few that the last link in the chain (a demonstration of the actual activity of these nuclear bodies as hereditary character bearers), due to the deficiencies in our knowledge of cell chemistry, has yet to be forged. This latter point is one about which geneticists and cytologists are, at present, not greatly concerned and conclusive data upon the physiological behavior of the chromosomes does not seem at all imminent. While information is gradually accumulating in preparation for this final analysis much may still be accomplished by purely morphological studies with tentative interpretations of physiological activity based on these observations. A recent study of the chromosomes associated with sex in the chick (5) involved extensive observations on the chromosomes of dividing somatic cells, the results of which are recorded below.

Observations.

The technique of preparation (3), of counting and measuring the chromosomes, the amount and source of the material (5) have all been recorded and need not be repeated here. The chromosomes of over 150 cells have been drawn and studied. As the nervous system is the most actively growing of all the regions of the body the majority of the cells studied have naturally been found in some part of it. Cells with clear polar views of metaphase chromosomes have been found in the brain, neural tube,

[1] The observations recorded in this paper were made principally at the University of Pennsylvania during the writer's tenure of a National Research Council Fellowship.

optic cup, auditory vesicle, connective tissue, heart muscle, blood, amnion, gonads and in tissue cultures of muscles.

The Chromosome Number.—The difficulties involved in determining the exact number of chromosomes in the chick have been described in detail (5). Briefly, the trouble encountered may be said to be due, first to the extreme smallness of the shortest chromosomes of the complex making observation at times uncertain and second, to the apparent failure of the component granules of the smaller chromosomes to unite or at least to clearly indicate their proper relations to each other until late metaphase if indeed it really and always happens then.

The average number of chromosomes in the soma of the chick, based on 78 counts, is 33. I think that this is perhaps lower than the actual number which the most satisfactory counts indicate to be about 35 or 36. This variation in the chick is apparently due to a failure of the chromomeres or parts of the smaller chromosomes to unite rather than to fragmentation as in the case of the pig (1). This opinion is based upon the observations in the chick of larger numbers of distinct chromatin bodies (with occasional visible connecting threads between them) in the prophase than could be found in cells in later stages of mitosis whereas in the pig a reduction in the chromosome number as the metaphase was neared did not occur. The smaller chromosomes of the chick are the only ones concerned while in the pig the long ones are the ones that become broken up.

The Chromosome Form.—All of the longer chromosomes (about 12 in number) of the somatic cells are in the form of J's while the shorter ones are rods. Their structure and size relations (5) are alike in all the tissues studied including tissue cultures (4). The largest chromosome (chromosome pair in the male) of the complex has been shown to be the one associated with sex and is found in all somatic cells as clearly as in the cells of the gonads (5), Figs. 1 to 9. The female embryonic cells are heterozygous for this chromosome while the male cells are homozygous. No differences in the chromosomes or their behavior have been noted between any of the body tissues or in comparison with those of the embryonic germ cells.

Discussion.

The morphological data on the behavior of the chromosomes in the developing embryo, although admittedly scant, has so far given no clue to the manner in which the chromosomes may contribute their potentialities to the growing organism. Before a study of somatic chromosomes had been made it seemed reasonable to expect to find the various highly differentiated cells of the body with chromosome numbers, morphology or behavior at variance both with those found in other tissues and with the specific number and general characteristics found in the gonads. This has been found not to be the case in at least three forms, the pig (1), the evening primrose (2) and the chick (5). There was some fragmentation of the somatic chromosomes in the first two forms but it was shown that none of the chromatin was lost. Furthermore this fragmentation was neither specific for any particular tissue nor constant in amount. In general the chromosome situation in the soma seems to be entirely similar to that found in the unreduced gonad cells. This is a matter difficult to understand in the present state of our information. Of all the characteristics that must be controlled or borne by or at least associated with the chromosomes (as shown by the studies on *Drosophila*) few have any chance for expression in the majority of the somatic cells and tissues and must therefore be inhibited in one way or another. The cells of the lower forms of animal life largely retain the germ-like power of reproducing the portion lost or even an entire organism following injury. This power is perhaps even more marked in plants. As differentiation becomes more extreme in the animal kingdom the ability to regenerate a part or a whole animal from cells already specialized becomes less and less until in the highest types of animals somatic cells are usually able to produce only somatic cells like themselves. Yet the chromosomes in these highly specialized cells have, in the examples studied, been found to be entirely similar to those in the germ cells containing the possibilities for a complex animal or plant. This may suggest that the chromosomes are functionless in the differentiated Soma. Or it may be that having contributed their share in the production of the specialized tissue are thereafter inactive as far as are concerned the general somatic

attributes or determiners with which originally they must have been equipped. If the latter interpretation is correct the perpetuation of the complete mitotic mechanism in the soma as it exists in the reproductive organs may seem, as far as any need for an exact division of the genes is concerned, somewhat unnecessary, to be classed possibly with vestigial organs and having no more significance. In view of the great delicacy of the mechanism that exists in all cells for accurately dividing the chromatin the last suggestion does not seem impressive. But as far as our morphological data on the behavior of somatic chromosomes go, together with the behavior of the soma in growth and regeneration, the above suggestion is at least a possibility to be considered in our attempt to get at the physiology of development and genetics.

Summary.

1. The chromosome number in the somatic cells of the chick is about 35 or 36.

2. No characteristic differences between the number, the sizes, the morphology or the behavior of the chromosomes in comparison either with each other or with the cells of the gonads have been noted.

3. In view of the entire similarity of the somatic and germinal mitotic behavior and in consideration of the complete inability of highly specialized cells to regenerate other than cells similar to themselves it is tentatively suggested as a basis for future discussion that the somatic chromosomes, as far at least as their genetic function is concerned, have either become functionless or their cytoplasmic environment is incapable of reacting to the possibilities presumably carried by them.

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DESCRIPTION OF PLATE I.

All figures were drawn at table level with a Zeiss 1.5 mm. apochromat and a 15 × Orthoscopic ocular. They are reproduced at the size originally drawn. All are polar views of metaphase plates in chick embryonic cells with the exception of Fig. 5.

FIG. 1. From optic cup. 35 chromosomes.

FIG. 2. From amnion. 30 chromosomes.

FIG. 3. From a male gonad. 39 chromosomes.

FIG. 4. From neural tube. 32 chromosomes.

FIG. 5. Late prophase in connective tissue cell grown in a tissue culture. 38 chromosomes.

FIG. 6. From heart muscle. 33 chromosomes.

FIG. 7. From brain. 35 chromosomes.

FIG. 8. From female gonad. 36 chromosomes.

FIG. 9. From neural tube. 35 chromosomes.

ROBERT T. HANCE.

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